3075-Pos Board B230
Towards Detergent Free Solubilization of Membrane Proteins into Nanodiscs: A Biophysical Study on the Interaction between Styrene Maleic Acid (SMA) Copolymers and Synthetic Phospholipid Vesicles
Stefan Scheidelaar, Martijn C. Koorengevel, Hans Meeldijk, Eefjan Breukink, Antoinette Killian.
Utrecht University, Utrecht, Netherlands.
Recently, it has been discovered that SMA copolymers are able to solubilize membrane proteins in the form of styrene maleic acid lipid particles (SMALPs) without the use of destabilizing detergents [1,2]. In order to understand this unique property of SMA we studied the solubilization of multilamellar vesicles (MLVs) of synthetic saturated phospholipids by SMA as function of acyl chain length and temperature. The rate of solublization was monitored by transmission experiments. It was found that SMA polymers are excellent phospholipid membrane solubilizers, that are able to solubilize below, at and above gel to crystalline liquid phase temperature, with most efficient solubilization close to and at the gel to liquid-crystalline phase transition temperature (Tm). The formation of ~10 nm diameter SMALPs, also referred to as lipid-nanodisks was verified by negative stain-transmission electron microscopy (TEM) and dynamic light scattering (DLS). Comparison with the recombinant apolipoprotein MSP1D1 showed that SMA is a significantly more potent membrane solubilizer than MSP1D1. Based on these and other results we developed a model for the mode of action of SMA that will be presented here.
Our study contributes to the fundamental knowledge about the molecular mode of action of SMA, which is essential to develop general methods to succesfully extract membrane proteins directly from their native environment in the form of lipid-nanodiscs. As proof of principle that this is indeed possible, we succeeded in purifying the potassium channel KcsA from the inner membrane of *E-coli* using the SMA technology. These results and some of its implications will be presented in an accompanying poster.
1. Knowles et al., 2009, JACS, 131, 7484-7485.
2. Orwick et al., 2012, Angew.Chem., 51,1-6

3076-Pos Board B231
Characterization of Anticancer Peptides in Membrane Disruption
Sai Janani Ganesan[1], Joel P. Schneider[2], Robert Blumenthal[2], Silvina Matysiak[1].
[1]University of Maryland, College Park, College Park, MD, USA, [2]National Cancer Institute, Frederick, MD, USA.
Cationic antimicrobial peptides have long been shown to disrupt membranes or display anticancer activity. A series of 18-20 residue anticancer peptides, with increasing hydrophobicities, have been designed to take advantage of the electronegativity of tumor cells, which arises from the aberrant lipid composition of the outer leaflet of the cell membrane 1. The designed anticancer peptides preferentially fold to a β-hairpin at the surface of cancer cells, and contain alternating valine (V) and lysine (K) residues, representing the N- and C- terminal strand regions, with a defined turn region, made of four amino acids (-VPPT-). We have studied the process of membrane disruption by the above mentioned anticancer peptides, using coarse grained MARTINI model and atomistic molecular dynamics simulations. We have also studied the mechanism of action of these peptides under various lipid compositions of phosphatidylserine and phosphatidylcholine. Our results suggest that the primary driving force for membrane disruption is the electrostatic interaction between the anionic phosphatidyserine and the cationic anticancer peptides.
1.Anticancer β-Hairpin Peptides: Membrane-Induced Folding Triggers Activity; Chomdao Sinthuvanich, Ana Salomé Veiga, Kshitij Gupta, Diana Gaspar, Robert Blumenthal, and Joel P. Schneider; Journal of the American Chemical Society 2012 134 (14), 6210-6217

3077-Pos Board B232
Complex Supported Lipid Bilayers with High Cholesterol Content formed by α-Helical Peptide-Induced Vesicle Fusion
Gregory J. Hardy.
Duke University, Durham, NC, USA.
The objective of this work is to establish α-helicial (AH) peptide-induced vesicle fusion as a reliable and facile technique to form supported lipid bilayers (SLBs) containing a high cholesterol content and multiple lipid types. Vesicles consisting of POPC : POPE : POPS : sphingomyelin : cholesterol (9.35 : 19.25 : 8.25 : 18.15 : 45.00) were used to form a SLB that models the native composition of the human immunodeficiency virus-1 (HIV-1) lipid envelope. In the absence of AH peptides, these biomimetic vesicles failed to form a complete SLB. Methods: We verified and characterized AH peptide-induced vesicle fusion by quartz crystal microbalance with dissipation monitoring (QCM-D), neutron reflectivity, and high-resolution scanning-probe imaging.
Our results showed that AH peptide-induced vesicle fusion is a reliable method to engineer supported lipid bilayers (SLBs) containing complex membrane compositions including a high concentration of cholesterol and membrane embedded peptides. Successful SLB formation entailed a QCM-D characteristic frequency shift of -35.4 ± 2.0 Hz and a change in dissipation energy of 1.91 ± 0.52 x 10-6. Neutron reflectivity measurements determined the SLB thickness to be 49.9 +1.9-1.5 Å, and showed the SLB to be 100 +0.0-0.1% complete and void of residual AH peptide after washing. Atomic force microscopy imaging confirmed complete SLB formation and revealed three distinct membrane domains with no visible defects.
Our research is significant in that it provides a biologically relevant system to screen protein-membrane interactions with a broad range of diagnostic tools. Given the success reported here and by Cho and coworkers in using AH peptide-induced vesicle fusion, there is potential for this technique to form SLBs under a range of conditions and surfaces that are generally unfavorable for spontaneous vesicle fusion.

3078-Pos Board B233
Influence of Histidine Residues on Transmembrane Helix Alignment
Ashley N. Martfeld, Denise V. Greathouse, Roger E. Koeppe II.
University of Arkansas, Fayetteville, AR, USA.
To investigate His residues in lipid bilayer membranes, we have employed GWALP23 (acetyl-GGALW^{5}LALALALALALW^{19}LAGA-amide) as a favorable host peptide. Importantly, membrane-spanning GWALP23 is quite sensitive to single-residue replacements, in part because the transmembrane helix exhibits only limited dynamic averaging of solid-state NMR observables such as the ^{2}H quadrupolar splitting (Biophys. J. 101, 2939). We inserted His residues into position 12 and/or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific ^{2}H-Ala labels within the helical core sequence. Solid-state ^{2}H NMR spectra reveal a marked difference between the L12H mutant and the L12H-A13H double mutant. GWALP23-H12 exhibits a well-defined tilted transmembrane orientation in both DOPC and DLPC bilayer membranes, suggesting that the bilayer-incorporated histidine side chain is neutral (deprotonated) at an experimental pH of 5-6. By contrast, GWALP23-H12,13 is highly dynamic and exhibits multiple states which are in slow exchange on the NMR timescale. Indeed, the multi-state behavior of GWALP23-H12,13 between pH 4 and pH 9 resembles closely that of GWALP23-R12 (J. Am. Chem. Soc. 132, 5803). Further aspects of the pH dependence of transmembrane helices having one or two histidines are under investigation.

Membrane Active Peptides & Toxins II

3079-Pos Board B234
Effect of Curcumin on Peptide-Membrane Interaction: In Kinetics and in Equilibrium
Wei-Chin Hung[1], Ming-Tao Lee[2,3], Chang-Chun Lee[4], Huey W. Huang[4].
[1]R.O.C. Military Academy, Fengshan, Taiwan, [2]National Synchrotron Radiation Research Center, Hsinchu, Taiwan, [3]National Central University, Jhongli, Taiwan, [4]Rice University, Houston, TX, USA.
We performed a three-chamber aspirated GUV experiment to study the competitive binding between curcumin and melittin. GUVs were produced and aspirated in chamber 1. Chamber 2 contained 10 microM curcumin. Chamber 3 contained 10 microM curcumin and various concentrations of peptides: penetratin, melittin or magainin. Aspirated GUVs were first equilibrated in chamber 2 and then transferred to chamber 3, where we observed the response of the GUV to the addition of peptides. Without the peptides in chamber 3, the GUV should remain unchanged. The results can be described as the peptides replacing curcumin bound on the bilayer interface and shifting curcumin into the hydrocarbon region, except for magainin. The bound curcumin essentially blocked magainin from binding to the membranes. This can be explained as a kinetic effect because the effect size of a bound magainin is twice the effective size of a bound curcumin, whereas the effective sizes of penetratin and melittin are comparable to curcumin (this is an usual property of curcumin for its small molecular size). Thus for penetratin and melittin, the behavior of the peptides is the same with or without curcumin. On the other hand, the curcumin/peptide/lipid mixtures produce bilayers totally unlike either curcumin with lipid or peptide with lipid. Instead of steady membrane thinning by either melittin or curcumin alone, the addition of melittin to curcumin/DOPC actually thickening the bilayers. This is the first example of incompatible results from equilibrium experiment and from kinetic experiment. It implies that competitive binding of two different membrane-active molecules can be complicated, a caution for drawing conclusion about synergy effects.